POP-4 SEPARATION POLYMER (3.5ML)

INSTRUCTIONS FOR USE

1. PRODUCT INFORMATION

Catalog Number	EV-SGR-005
Product Name	POP-4 Separation Polymer (3.5ml)
Category	CE Separation Polymer
Pack Size	3.5ml/bottle
Regulatory Status	For Research Use Only (RUO)
OEM Reference	Contact techsupport@enzovera.com
Version	1.0
Issue Date	2026-05-07

2. INTENDED USE

This product is intended for use as a separation polymer in capillary electrophoresis on Applied Biosystems 3100, 3130, and 3500 series genetic analyzers. POP-4 Separation Polymer is optimized for fragment analysis applications including microsatellite analysis, AFLP, and short-read DNA sequencing up to 550 bases. This ready-to-use formulation provides consistent separation performance and is compatible with standard capillary array configurations. For Research Use Only. Not for use in diagnostic procedures.

3. KIT COMPONENTS

Component	Quantity / Volume	Storage
POP-4 Separation Polymer	1 bottle × 3.5 mL	2-8°C, protected from light
Polymer Conditioning Solution	1 vial × 2.0 mL	2-8°C
Capillary Storage Solution	1 vial × 5.0 mL	15-30°C
Syringe Filter (0.2 µm PVDF)	2 filters	15-30°C
Septum Caps	5 caps	15-30°C
Quick Reference Protocol	1 card	Room temperature

4. MATERIALS REQUIRED BUT NOT PROVIDED

- Applied Biosystems 3130/3130xl/3730/3730xl Genetic Analyzer or equivalent capillary electrophoresis system
- 50 cm capillary array appropriate for instrument model
- Anode buffer container and cathode buffer container
- 1X running buffer (contact Enzovera Technical Support at techsupport@enzovera.com for recommended formulation)
- Sequencing reaction products prepared with BigDye Terminator chemistry or equivalent
- Septum and septa retainer appropriate for instrument model
- Clean, lint-free laboratory wipes for instrument maintenance
- Waste container for polymer disposal

5. STORAGE AND STABILITY

Storage Temperature	-20°C, protect from light
Appearance	Viscous clear polymer solution
Shelf Life	12 months from manufacture date
Shipping Conditions	On dry ice
Freeze-Thaw Cycles	Maximum 3 cycles recommended
Working Solution	Stable on ice for up to 8 hours

6. PRECAUTIONS AND WARNINGS

- For Research Use Only. Not for use in diagnostic procedures.
- Avoid repeated freeze-thaw cycles. Aliquot reagents if needed.
- Handle all reagents on ice. Return to -20°C storage immediately after use.
- Wear appropriate PPE: gloves, lab coat, and eye protection at all times.
- Dispose of waste in accordance with local, state, and federal regulations.
- Do not use reagents past their expiry date.

7. PROTOCOL

PROTOCOL FOR POP-4 SEPARATION POLYMER (3.5mL)

ENZOVERA LIFE SCIENCES

For Research Use Only

PRODUCT: POP-4 Separation Polymer

CATALOG NUMBER: Contact Enzovera Technical Support at techsupport@enzovera.com

LOT NUMBER: Lot-specific

VOLUME: 3.5 mL per syringe

INSTRUMENTS: ABI PRISM 3100/3100-Avant, 3130/3130xl, 3500/3500xL Genetic Analyzers

APPLICATIONS: Fragment analysis (15-500 bp), microsatellite genotyping, short-read Sanger sequencing

STORAGE AND HANDLING

Store POP-4 polymer syringes at 2-8°C protected from light. Do not freeze. Bring polymer to room temperature (20-25°C) for 30 minutes before use. Inspect syringe for air bubbles or particulates before installation. Discard if polymer appears cloudy or discolored. Shelf life is 12 months from date of manufacture when stored properly.

CAPILLARY ARRAY PREPARATION

1. Remove the capillary array from the instrument according to manufacturer instructions. Inspect capillaries for damage, scratches, or polymer residue on the exterior surface.

2. If performing a first-time installation or after extended storage (>7 days), condition new capillaries by filling with deionized water. Run a water wash for 20 minutes at 20°C to remove storage buffer and residual manufacturing materials.

3. Flush capillaries with 1X running buffer (cathode buffer) for 10 minutes at 50°C to equilibrate the capillary walls and establish baseline electroosmotic flow conditions.

4. Dry capillaries by applying positive pressure (nitrogen or compressed air at 40-60 psi) for 2 minutes to remove all liquid. This step is critical to prevent polymer dilution and ensure proper viscosity.

POLYMER SYRINGE INSTALLATION

5. Remove POP-4 syringe from refrigerated storage and allow to equilibrate to room temperature for 30 minutes. Do not attempt to use cold polymer as viscosity will be too high for proper delivery.

6. Inspect the syringe barrel for air bubbles. If bubbles are visible, gently tap the syringe vertically with the tip pointing upward to move bubbles toward the plunger end. Do not shake vigorously as this introduces additional bubbles.

7. Remove the syringe cap slowly to avoid creating negative pressure that draws air into the polymer. Attach the syringe to the polymer delivery block on the instrument according to manufacturer specifications.

8. Prime the polymer block by advancing the syringe plunger slowly until a small bead of polymer appears at the capillary interface port. This confirms continuity and removes air from the connection pathway.

CAPILLARY FILLING PROCEDURE

9. Install the prepared capillary array onto the instrument detection stage. Ensure the array is properly seated and the detection window is aligned with the laser optics.

10. Position the array inlet end into the polymer block interface. Ensure a tight seal to prevent air entrainment during the filling process.

11. Initiate the polymer fill using instrument software. For ABI 3100/3130 series instruments, use the standard fill protocol with 900 seconds duration at 20°C. For 3500 series instruments, use 420 seconds duration per manufacturer guidelines.

12. Apply positive pressure (instrument-controlled pneumatic system) to drive polymer into the capillaries. Typical fill pressure is 40-50 psi for 36 cm capillaries and 50-60 psi for 50 cm capillaries.

13. Monitor polymer fill progress on instrument display. Successful fill is indicated by stable pressure readings and polymer emerging from capillary outlets into the cathode buffer reservoir.

14. After filling is complete, carefully remove the capillary array from the polymer block and position the inlet ends into the anode buffer reservoir containing fresh 1X running buffer.

CAPILLARY CONDITIONING AND EQUILIBRATION

15. Perform a pre-run conditioning step by applying 15 kV for 3 minutes with capillary tips submerged in running buffer. This aligns polymer chains and establishes stable current.

16. Monitor baseline current stability. Acceptable current range is 6-10 microamperes for 36 cm capillaries and 4-8 microamperes for 50 cm capillaries at 15 kV. Current outside this range indicates incomplete filling or buffer contamination.

17. Run a spectral calibration using the instrument's dye set calibration protocol. For fragment analysis applications using DS-33 matrix, inject calibration dye mix for 5 seconds at 1.2 kV and separate at 15 kV for 45 minutes at 60°C.

18. Verify separation performance by running a sizing standard such as GeneScan 500 LIZ or MapMarker 1000. Peak resolution should be baseline-separated with symmetrical Gaussian peak shapes.

SAMPLE INJECTION AND SEPARATION

19. Prepare samples according to standard protocols for fragment analysis or sequencing applications. For fragment analysis, dilute

8. EXPECTED RESULTS

Under standard operating conditions on ABI 3100/3130/3500 instruments, POP-4 Separation Polymer delivers baseline resolution of DNA fragments from 50-500 bp with <0.5 bp sizing accuracy when used with appropriate size standards. Sequencing applications typically achieve read lengths of 500-650 bases with QV20 accuracy at default run parameters (injection voltage 1.6 kV, injection time 15 sec, run voltage 15 kV). Performance metrics are equivalent to Applied Biosystems POP-4 reference polymer, with

reproducible peak heights >1000 RFU for standard DNA concentrations and minimal baseline noise (<5 RFU).

9. TROUBLESHOOTING GUIDE

Problem	Possible Cause	Recommended Action
Poor peak resolution or broad peaks	Polymer degradation due to improper storage, expired product, or contamination with salts or particulates	Verify polymer is stored at 4°C and protected from light. Check expiration date. Use fresh polymer aliquot. Ensure capillaries are clean and properly conditioned. Pre-run new polymer for 5 minutes at 15 kV before sample injection.
High background or baseline noise	Contaminated polymer, residual buffer salts in capillary, or fluorescent debris from previous runs	Replace polymer with fresh aliquot. Perform capillary wash sequence: 1M NaOH (2 min), deionized water (2 min), fresh POP-4 (5 min). Ensure all buffers and reagents are free from particulates. Filter polymer through 0.2 µm filter if debris is visible.
Short read lengths or premature signal dropout	Insufficient polymer viscosity, air bubbles in polymer syringe or capillary, or incomplete capillary filling	Centrifuge polymer bottle at 2000 × g for 5 minutes to remove air bubbles before loading syringe. Fill capillary slowly (60 seconds per 36 cm capillary). Verify polymer syringe seal is intact. Check capillary for blockages or damage. Ensure instrument temperature is stable at 50-60°C during run.
Dye blob artifacts or mobility shifts	Inadequate sample cleanup, excess dye terminators, or polymer-dye interaction from repeated injections	Perform thorough post-reaction cleanup using Enzovera CleanSeq magnetic beads or ethanol precipitation. Verify cleanup removes >99% unincorporated dye terminators. Replace polymer after every 96-well plate or when artifacts appear. Use fresh capillary if mobility shifts persist after polymer replacement.
Inconsistent run-to-run performance	Temperature fluctuations during runs, polymer lot variation, or capillary conditioning inconsistencies	Verify instrument oven temperature stability (±0.5°C). Equilibrate new polymer lot to room temperature (15 minutes) before use. Standardize capillary conditioning protocol: pre-run polymer at 15 kV for 5 minutes for first injection, then 3 minutes between runs. Store opened polymer bottles at 4°C and use within 30 days.
No signal or very weak fluorescence	Blockage in capillary, polymer not entering capillary, incorrect optical detection settings, or	Inspect capillary windows for cracks or coating damage. Verify polymer viscosity (polymer

	sample not injecting	should flow slowly, not watery). Check injection voltage and time settings (typically 1-3 kV for 10-30 seconds). Confirm laser alignment and PMT sensitivity settings on instrument. Replace capillary array if blockage cannot be cleared with 1M NaOH wash.

10. DOCUMENT CONTROL

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